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Dissociation of Coronary Vascular Tolerance and Neurohormonal Adjustments During Long-Term Nitroglycerin Therapy in Patients With Stable Coronary Artery Disease

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Objectives. **We sought to examine whether long-term nitroglycerin treatment causes tolerance in large coronary arteries and whether the loss of vascular effects parallels neurohormonal adjustments.**

Background. **Nitroglycerin therapy is associated with increased plasma renin activity and aldosterone levels and a decrease in hematocrit. It is assumed that nitroglycerin tolerance results in part from these neurohormonal adjustments and intravascular volume expansion.**

Methods. **Three groups were studied: group I (n = 10), no prior nitroglycerin therapy; and group II (n = 10) and group III (n = 8), 24- and 72-h long-term nitroglycerin infusion (0.5 μg/kg body weight per min), respectively. Coronary artery dimensions were assessed using quantitative angiography. Plasma renin activity, plasma aldosterone and vasopressin levels and hematocrit were monitored before and during nitroglycerin infusions.**

Results. **In group I, increasing intravenous concentrations of nitroglycerin caused a dose-dependent increase of the midportion of the left anterior descending coronary artery (baseline diameter 2.13 ± 0.07 mm [mean ± SEM], maximally by 22 ± 2%) and left circumflex coronary artery (baseline diameter 2.08 ± 0.07 mm, maximally by 22 ± 3%). An intracoronary nitroglycerin bolus (0.2 mg) caused no further significant increase in diameter, indicating maximal dilation. In group II (n = 10), the baseline large coronary artery diameter under ongoing nitroglycerin was significantly larger than that in group I (left anterior descending artery 2.61 ± 0.08 mm, left circumflex artery 2.57 ± 0.08 mm). Additional intravenous and intracoronary nitroglycerin challenges did not cause further dilation, indicating maximally dilated vessels. At the same time, plasma renin activity, plasma aldosterone and vasopressin levels were significantly increased, and hematocrit significantly decreased. In group III patients, the baseline diameter of the left anterior descending artery and the left circumflex artery did not differ from that in patients without nitroglycerin pretreatment, indicating a complete loss of nitroglycerin coronary vasodilative effects. These patients showed no significant increase in circulating neurohormonal levels but a significant decrease in hematocrit.**

Conclusions. **Within 24 h of continuous nitroglycerin treatment, the coronary arteries were maximally dilated despite neurohormonal adjustments and signs of intravascular volume expansion. Within 3 days of nitroglycerin infusion, tolerance developed in the absence of neurohormonal activation. The dissociation of neurohormonal adjustments and tolerance in large coronary arteries indicates that after long-term nitroglycerin treatment, true vascular tolerance, perhaps from an intracellular tolerance step, may have developed.**

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The efficacy of nitroglycerin in the treatment of ischemic heart disease is dependent on its unique spectrum of in vivo activity: Nitroglycerin preferentially dilates large arteries, capacitance vessels and collateral channels, with little measurable effect on systemic and coronary vascular resistance (1). However, during long-term exposure the anti-ischemic efficacy of nitroglycerin is rapidly blunted by the development of tolerance (2–4). Several mechanisms have been suggested to account for this phenomenon. Mechanisms extraneous (so-called pseudotolerance) to the vessel wall include neurogenic counterregulatory mechanisms (2,3) and intravascular volume expansion (5). Neurogenic counterregulatory mechanisms may offset the direct vascular effects of nitroglycerin and, together with sodium and water retention, may counterbalance the venodilator effects of this drug. In experimental animals, tolerance has also been shown to be related to an inability of the vascular smooth muscle to convert nitroglycerin to nitric oxide (so-called true vascular tolerance [6–8]). There has been substantial debate on the extent to which these two mechanisms contribute to the loss of antianginal efficacy during long-term nitroglycerin therapy, and the notion that neurohumoral mechanisms are a predominant cause has achieved substantial popularity (2,3).

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This issue is confounded by the multiple effects of nitroglycerin and the various variables that may be examined. Studies that examine only peripheral consequences of nitroglycerin therapy may not detect changes in coronary vascular responses to nitroglycerin. Therefore, the aim of the present study was 1) to determine whether the nitroglycerin-induced dilation of large epicardial arteries is subject to tolerance under long-term nitroglycerin treatment; and 2) to evaluate the effects of continuous nitroglycerin treatment on neurohormonal variables to establish whether the reflex activation of neurohormonal vasoconstrictor forces is able to attenuate the nitroglycerin coronary vasodilator effects.

Methods

Patients. The subjects were patients undergoing routine diagnostic cardiac catheterization for evaluation of chest pain. Patients with unstable angina, recent myocardial infarction (<1 month), significant valvular heart disease or clinical evidence of heart failure were excluded. The study protocol was approved by the ethics committee of the University of Freiburg. Written informed consent was obtained from all patients. We evaluated three groups of patients with suspected coronary artery disease. *Group I* included 10 patients without nitroglycerin pretreatment (9 men, 1 woman; mean ± SEM age 53 ± 4 years). Diagnostic catheterization revealed significant coronary artery disease (defined as >50% stenosis in one or more vessels) in eight patients (four with two-vessel disease, four with one-vessel disease). Two patients had normal coronary arteries. *Group II* included 10 patients (8 men, 2 women; mean age 56 ± 4 years) pretreated with nitroglycerin for 24 h (0.5 μg/kg body weight per min). Two patients in group II had normal coronary arteries, and eight patients had significant coronary artery disease (one with three-vessel disease, three with two-vessel disease, four with one-vessel disease). Group III included eight patients (seven men, 1 woman; mean age 52 ± 3 years) who had received nitroglycerin 0.5 μg/kg per min for 72 h (two patients with two-vessel disease; five with one vessel disease). One patient had normal coronary arteries, and seven had significant coronary artery disease.

Study design. Antianginal medications (beta-adrenergic blocking agents, calcium antagonists and long-acting nitrates) were discontinued at least 24 h before the study. All studies were done in the morning between 8 and 11 AM. The experimental protocol was performed after the left heart catheterization with the subjects in the fasting state and premedication with 10 mg of oral diazepam.

After baseline measurements, nitroglycerin was given to patients without nitroglycerin pretreatment in the following sequence: 1) 0.05 μg/kg per min intravenously for 7 min; 2) 0.15 μg/kg per min intravenously for 7 min; 3) 0.5 μg/kg per min intravenously for 7 min; 4) 200 μg into the left main stem to assess the maximal vasodilator capacity of the left epicardial coronary arteries.

Throughout the study, aortic pressure and heart rate were continuously monitored. Nitroglycerin was infused intravenously with an infusion pump (Braun Melsungen) set at a flow rate of 2 ml/min. At the end of each infusion (and also 5 min after the intracoronary nitroglycerin bolus), biplane coronary angiograms were taken. After a dose–response relation for intravenous nitroglycerin and large coronary artery dilation had been established in patients without nitroglycerin pretreatment, patients were randomly assigned either to the 24-h (group II) or 72-h nitroglycerin treatment group (group III), where the identical protocol was performed. For long-term infusion, nitroglycerin at 0.5 μg/kg per min was chosen, because this particular concentration caused a maximal dilation of the left anterior descending and left circumflex coronary arteries (Fig. 1). Throughout the protocol for groups II and III, long-term nitroglycerin infusion was continued.

Quantitative coronary angiography. Coronary angiography was done with a simultaneous biplane multidirectional isocentric radiographic system (Siemens Bicor). End-diastolic cine frames of biplane cineangiograms were videodigitized and stored in a Mipron I image analysis system (Kontron Electronics) in a 512 × 512 matrix with an eight-bit gray scale as described previously (10). Quantitative coronary angiography of midsegments of the left circumflex and left anterior descending coronary arteries was done by automatic contour detection, which was validated by a method that incorporates a geometric edge differentiation technique. If proximal left circumflex artery or left anterior descending artery stenosis was present, segments distal to the stenosis were analyzed. Calculation of the exact radiologic magnification factor of the measured segment was used to scale the data from pixels to millimeters as previously described (11). The accuracy and precision of this technique, as well as the reproducibility of serial measurements under routine clinical conditions, have been established (10). The evaluation of the angiograms was done by a technician who was unaware of the patients' pretreatment conditions. Plasma samples for analysis of aldosterone, vasopressin and plasma renin activity were obtained in a supine position after a 30-min rest period and 1 h before diagnostic catheterization. In patients treated with nitroglycerin for 24 or 72 h, blood samples were drawn at a similar time of day. Plasma renin activity and plasma aldosterone and vasopressin levels were determined as described recently (9).

We also performed additional studies to exclude a confounding contribution of coronary artery diameter variability to our data. To address that issue, we also analyzed midsegments of the left anterior descending coronary artery by quantitative coronary angiography in 20 patients of similar age (55 ± 2 years) and with a similar degree of coronary artery disease (8 with two-vessel disease, 10 with one-vessel disease, 2 without significant coronary artery stenosis).

Statistical analysis. All data are expressed as mean value ± SEM. For within-group comparisons, a one-way analysis of variance for multiple comparisons was applied, followed by a *t* test with Bonferroni's correction for the number of comparisons. For direct comparison of the overall dose–response relation with respect to large coronary artery diameter and systemic hemodynamic variables, an analysis of co-

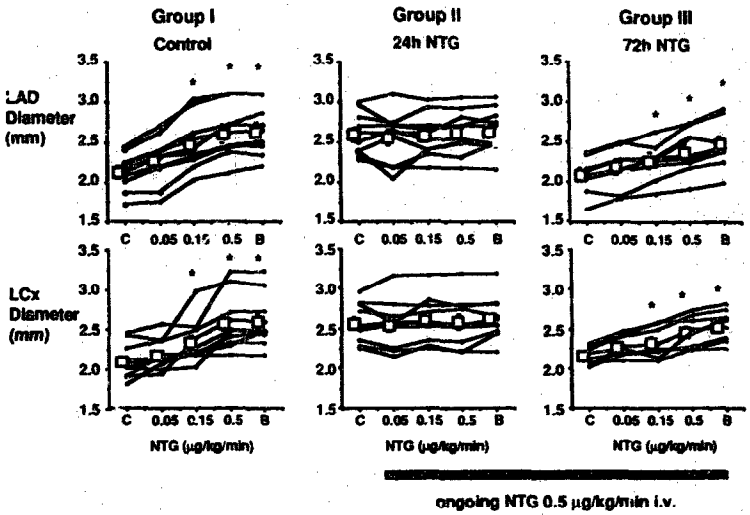

Figure 1. Effects of increasing intravenous (i.v.) and intracoronary nitroglycerin (NTG) on large coronary artery diameters in patients without (group I) and with 24 h (group II) and 72 h nitroglycerin pretreatment (group III). Under ongoing nitroglycerin infusions, patients pretreated with nitroglycerin for 24 h did not respond with a further increase in diameter, indicating a maximally dilated coronary artery. In contrast, patients pretreated with nitroglycerin for 3 days responded with a diameter increase that was not statistically different from those of group I, which is strongly suggestive of tolerance development in large epicardial arteries. Increasing concentrations of nitroglycerin were given intravenously for 7 min each. C = control; B = 0.2-mg intracoronary nitroglycerin bolus; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery. Data shown are mean value ± SEM (**open squares**) and individual data. *Significantly different from baseline values (after Bonferonni correction for the numbers of comparisons [n = 4]).

variance was performed. The variables were treatment and baseline coronary artery diameter, baseline mean arterial pressure or baseline heart rate, respectively. Baseline diameters of all three groups were compared using one-way analysis of variance. To analyze the effects of nitroglycerin treatment on neurohormonal variables, comparisons were made by a paired *t* test; $p < 0.05$ was considered significant.

Results

Effects of nitroglycerin on large epicardial artery diameter. In patients not pretreated with organic nitrates, an intravenous nitroglycerin infusion (0.05, 0.15 and 0.5 µg/kg per min) caused a dose-dependent increase in the diameter of the midportion of the left anterior descending coronary artery (baseline 2.13 ± 0.07) by 7 ± 1%, 16 ± 3% and 23 ± 2% and the left circumflex coronary artery (baseline 2.08 ± 0.07 mm) by 5 ± 2%, 12 ± 3% and 22 ± 3% (Fig. 1). Intracoronary bolus injection of nitroglycerin 0.2 mg did not produce additional significant dilation (left anterior descending artery 24 ± 2% vs. baseline; left circumflex artery 23 ± 3% vs. baseline), indicating that intravenous nitroglycerin infusion in a concentration of 0.5 µg/kg per min intravenously was sufficient to cause maximal dilation of large epicardial conductance vessels in normotensive patients.

In patients pretreated with nitroglycerin for 24 h (group II), the average baseline diameter for the left anterior descending artery (2.61 ± 0.08 mm) and the left circumflex artery (2.57 ± 0.08 mm) were significantly ($p = 0.01$ and 0.012, respectively) higher than the average baseline values in group I. Additional intravenous and intracoronary nitroglycerin challenges did not lead to further significant changes of the diameter of the midportion of the left anterior descending artery (maximally 1 ± 1%, ns) or the left circumflex artery (maximally 2 + 1%).

In contrast, the baseline diameter of the left anterior descending artery (2.10 ± 0.08 mm) and the left circumflex artery (2.16 ± 0.04 mm) of patients treated with nitroglycerin for 72 h (group III) did not differ significantly ($p = 0.973$ and 0.835, respectively) from baseline values of patients without nitroglycerin treatment (group I) and were significantly smaller than those in patients treated with nitroglycerin for 24 h ($p = 0.011$ and 0.010, respectively). Additional short-term challenges with intravenous nitroglycerin at 0.05, 0.15 and 0.5 µg/kg per min and intracoronary nitroglycerin at 0.2 mg under ongoing nitroglycerin (0.5 µg/kg per min) caused a dose-dependent increase in coronary artery diameter similar to that in group I (left anterior descending artery by 4 ± 1%, 8 ± 1%, 14 ± 2% and 18 ± 2%; left circumflex artery by 5 ± 1%, 9 ± 2%, 14 ± 2% and 17 ± 2%).

The overall dose–response relation for large coronary artery dilation for groups I and III was not significantly different ($p = 0.96$). In contrast, there were significant differences between groups I and II ($p = 0.020$) and between groups II and III ($p = 0.021$). The calculated ED_{50} (nitroglycerin concentration that causes half-maximal dilation of the left anterior descending artery) for group III was 0.66 ± 0.02 µg/kg per min and therefore significantly ($p = 0.01$) higher compared with that for patients without nitroglycerin pretreatment (0.11 ± 0.02 µg/kg per min).

In separate studies we analyzed the diameter of the midsegment of the left anterior descending coronary artery in 20 patients with coronary artery disease. The diameter of the left anterior descending coronary artery averaged 2.18 ± 0.05 mm and was therefore not significantly different from diameters in

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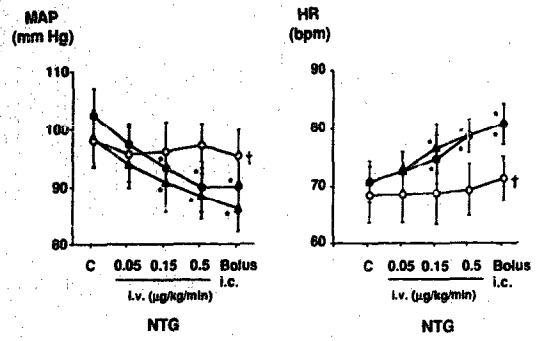

Figure 2. Effects of increasing intravenous (i.v.) and intracoronary (i.c.) nitroglycerin (NTG) concentrations on heart rate (HR) and mean arterial pressure (MAP) in patients without (group I **[solid circles]**) and with nitroglycerin pretreatment for 24 h (group II **[triangles]**) or 72 h (group III **[open circles]**). Changes in systemic hemodynamic variables in response to nitroglycerin were similar in groups I and II. However, the overall dose–response relation was significantly blunted in patients treated with nitroglycerin for 72 h. Bolus = 0.2-mg intracoronary nitroglycerin bolus; bpm = beats per minute. Data are expressed as mean value ± SEM. *Significantly different from control (C) values (after correction for the number of comparisons [n = 4]). †$p < 0.05$ versus overall hemodynamic response of groups I and II.

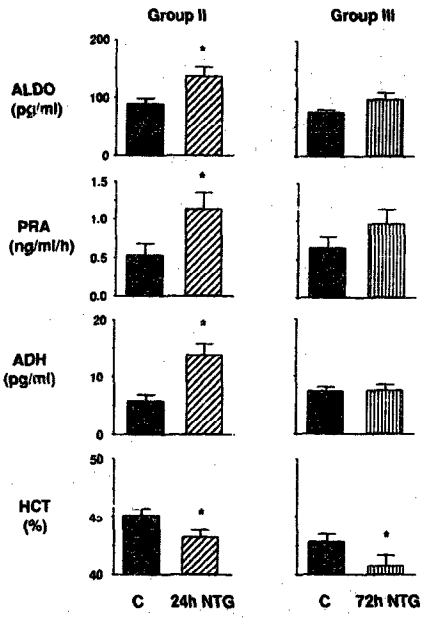

Figure 3. Effects of 24-h and 72-h nitroglycerin infusion, respectively, on plasma renin activity (PRA), plasma aldosterone levels (ALDO), plasma vasopressin levels (ADH) and hematocrit (HCT). Data are presented as mean value ± SEM. *$p < 0.05$ versus control values (C).

patients from groups I and III; however, it was significantly different from diameters in patients included in group II. Intracoronary bolus injection of nitroglycerin in a concentration of 0.2 mg in 11 patients increased the diameter of the midsegment of the left coronary artery diameter from 2.15 ± 0.08 to 2.68 ± 0.8 mm; the latter was not different from diameters in patients receiving ongoing NTG infusion for 24 h (group II).

Systemic hemodynamic variables. In patients without nitroglycerin pretreatment (group I) (Fig. 2), increasing concentrations of nitroglycerin caused a dose-dependent increase in heart rate (baseline 70 ± 4 beats/min) by maximally +15 ± 4% and drop in mean arterial pressure (baseline 102 ± 5 mm Hg) by maximally −12 ± 3%. In patients treated with nitroglycerin for 24 h (group II), short-term nitroglycerin challenges resulted in similar changes in systemic hemodynamic variables (heart rate: group I vs. group II, $p = 0.95$ [NS]; mean arterial pressure: group I vs. group II, $p = 0.43$ [NS]). In patients treated with nitroglycerin for 72 h the overall response in heart rate ($p = 0.014$) and mean arterial pressure ($p = 0.034$) was significantly attenuated compared with that in group I (Fig. 2).

Effects of nitroglycerin infusion on neurohormonal variables and plasma hematocrit. Intravenous nitroglycerin infusion for 24 h significantly increased plasma renin activity ($p = 0.03$) and plasma aldosterone ($p = 0.02$) and vasopressin ($p = 0.007$) levels (group II), (Fig. 3). Neurohormonal activation was accompanied by a significant drop in plasma hematocrit ($p = 0.01$), indicating intravascular volume expansion. In group III, neurohormonal activity after a 72-h nitroglycerin infusion period did not differ significantly from control values, whereas hematocrit was significantly ($p = 0.001$) reduced.

Discussion

The present study provides important information regarding the time course and mechanisms of nitrate tolerance during long-term intravenous nitroglycerin infusion. To our knowledge, this is the first demonstration that activation of circulating neurohormonal vasoconstrictor forces during the first 24-h nitroglycerin treatment period does not attenuate its coronary vasodilator effects. In contrast, after 3 days of nitroglycerin treatment, tolerance in both large epicardial arteries and the systemic vasculature develops without further signs of activation of circulating neurohormones. Considered together, the dissociation between neurohormonal adjustments and vascular tolerance in coronary arteries suggests that during the early phase of nitroglycerin infusion, neurohormonal activation does not attenuate large coronary artery vasodilation to nitroglycerin. However, after 3 days of continuous treatment, tolerance in large coronary arteries developed, as evidenced by a return to baseline size and a return of responsiveness to large doses of additional nitroglycerin. Because this occurred in the absence of further neurohumoral adjustments, it is likely that it was largely a result of intrinsic abnormalities of the tolerant vasculature.

A major aspect of the present study is the means used to define large coronary artery tolerance to nitroglycerin. As an index of nitroglycerin tolerance, we used the capacity of infusions of large concentrations of nitroglycerin superimposed on ongoing nitroglycerin treatment to cause further

vasodilation. We reasoned that additional higher concentrations of nitroglycerin would be unable to vasodilate vessels maximally dilated by nitroglycerin, whereas vessels in which the nitroglycerin effect was lost because of tolerance would be dilated by larger amounts of the drug. This assumption is based on extensive data from previous studies in animals and humans showing that high concentrations of nitroglycerin can consistently dilate coronary arteries tolerant to lower doses of the drug (12–15).

Limitation of the study. One potential criticism of this work is that the baseline diameters of the vessels studied varied among the three groups of patients. This seems unlikely for two reasons: 1) A widely accepted approach to estimating passive diameter (in the absence of tone) is to obtain measurements after the vessel is maximally dilated by nitroglycerin. This, in fact, was the measurement obtained after bolus injections of nitroglycerin in all three groups. Examination of Figure 1 shows that after a bolus injection of nitroglycerin, the coronary diameters were remarkably similar among the three groups of patients. This finding indicates that the decrease in baseline diameter observed in group III did not result from an intrinsic difference in the size of the vessel but from an increase in tone (likely related to loss of the effect of the nitroglycerin infusion effect). 2) In an additional 20 patients with similar risk profile, age and degree of coronary artery disease, we studied the baseline diameter of the midsegment of the left anterior descending artery. The average diameter did not differ significantly from the average diameter of patients without nitroglycerin pretreatment or of patients treated with nitroglycerin for 3 days. Furthermore, intracoronary bolus injections in 11 patients resulted in an average coronary artery diameter that was not different from that from patients under ongoing nitroglycerin infusion for 24 h. On the basis of these results, we believe that the larger diameters in the 24-h group compared with either the control group or the 72-h nitroglycerin infusion group resulted from a continued effect of nitroglycerin that is subsequently lost at 72 h.

Tolerance in large coronary arteries: pseudotolerance versus true vascular tolerance. The anti-ischemic effects of organic nitrates are rapidly blunted during continuous treatment with nitroglycerin infusion (2,4,16), isosorbide dinitrate (17) or nitroglycerin patches (18). Zimrin et al. (4) reported a marked attenuation of exercise tolerance in patients with stable coronary artery disease treated with nitroglycerin for 24 h. They speculated that activation of neurogenic vasoconstrictor forces might override the nitroglycerin vascular effects. The hypothesis that pseudotolerance mechanisms play a substantial role in nitrate tolerance is also supported by clinical data demonstrating that a nitrate-free interval prevents neurohormonal adjustments and parallel tolerance development (2). Furthermore, a causal role of neurohormones in nitroglycerin tolerance is indirectly supported by observations that concomitant administration of inhibitors of angiotensin-converting enzyme are in some but not all instances able to reverse or prevent tolerance (19–22). To address the eventual role of neurohormonal adjustments in nitrate tolerance of large epicardial arteries, we examined responses of these vessels to increasing doses of nitroglycerin and measured plasma aldosterone, plasma vasopressin and plasma renin activity before and during 24 to 72 h nitroglycerin infusion. As a marker of intravascular volume expansion, hematocrit was determined. The nitroglycerin concentration used for long-term infusion (0.5 μg/kg per min) was sufficient to cause maximal dilation of the left coronary artery, and the degree of diameter increase of the left anterior descending artery and the left circumflex artery (maximally +24%) is comparable to that reported after intracoronary nitroglycerin administration (23).

In the present studies we were able to demonstrate that within 24 h of continuous nitroglycerin treatment, the large coronary conductance vessels are still maximally dilated. This conclusion was based on observations that during continuous nitroglycerin infusion, the baseline diameters of the left coronary artery of group II were significantly higher than baseline values of control patients and did not differ from the maximally dilated diameters in group I. The maximal dilation observed after 24 h of nitroglycerin infusion indicated a continued nitroglycerin effect explaining the lack of effect of additional intravenous and intracoronary nitroglycerin challenges on left anterior descending and left circumflex artery (Fig. 1).

At the same time, we observed an increase in plasma renin activity, plasma aldosterone and vasopressin levels and a decrease in hematocrit. These changes represent neurohormonal adjustments and, as previously reported, are secondary to the hypotensive action of nitroglycerin (3,4) and other vasodilators (24,25). The lack of tolerance in large epicardial arteries provides direct evidence that, similar to experimental findings (15,26,27), activation of circulating neurohormonal vasoconstrictor forces does not impair nitroglycerin induced dilation of large coronary arteries.

After 72 h of continuous nitroglycerin treatment, the baseline diameter of the left circumflex artery or the left anterior descending artery under ongoing nitroglycerin infusion did not differ significantly from the baseline diameters of patients without nitroglycerin treatment, indicating an almost complete loss of coronary vasodilation.

This time course of nitroglycerin tolerance development in large coronary arteries is in keeping with recent experimental (13,15,28) and clinical data (12). During long-term nitroglycerin or isosorbide dinitrate infusion, the coronary arteries were maximally dilated 24 h after initiation of a 5-day nitroglycerin infusion period, and tolerance was well established by the third to fifth day of treatment.

Another important finding of the present study is that vascular tolerance after 72-h nitroglycerin infusion is not associated with further evidence of neurohumoral activation (Fig. 3). At this time, plasma aldosterone and vasopressin levels and plasma renin activity did not differ from values before nitroglycerin treatment. A similar transient pattern of neurohormonal adjustments during long-term nitroglycerin treatment was recently reported (3). In this previous study, treatment of healthy volunteers with nitroglycerin patches led to an increase in plasma renin activity and plasma aldosterone

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and vasopressin levels (comparable to group II) during the first day of treatment. In patients treated with nitroglycerin for 3 days, however, all three variables had returned to baseline (comparable to group III).

The demonstration of tolerance in large epicardial arteries in the absence of further increased neurohormonal activity makes it very unlikely that pseudotolerance mechanisms are responsible for that phenomenon. Normalization of circulating angiotensin II, aldosterone and vasopressin levels within 72 h of continuous nitroglycerin treatment, however, does not completely exclude that neurohormonal factors (e.g., plasma catecholamines) may limit nitroglycerin's coronary vasodilator effect at this time point. Plasma levels of these substances do not reflect changes in hormone turnover rates, neurohormonal clearance rates or the density of the respective receptors involved. Furthermore, a return of neurohormonal levels toward normal values may reflect a new homeostatic balance rather than normal neurohormonal activity. Notwithstanding these arguments, we believe that the attenuated activity of circulating neurohormones after a 3-day nitroglycerin treatment indicates that in addition to continued volume expansion, true vascular tolerance may have developed. This in turn may diminish baroreflex stimulation and subsequently neurohormonal activation.

In contrast to the transient changes in neurohormonal variables, we observed a consistent drop in hematocrit in patients treated with nitroglycerin for 72 h. A decrease in hematocrit during long-term nitroglycerin treatment is in agreement with previous observations and very likely reflects intravascular volume expansion secondary to a transvascular shift of fluid as a result of an alteration in Starling forces or a phenomenon related to an aldosterone-mediated salt and water retention (3,5,19). Recently, Parker and Parker (19) suggested that plasma expansion plays a more important role than neurohormonal responses in the loss of nitrate effects. During therapy with transdermal nitrates, they observed only modest and transient changes in neurohormonal variables, whereas, similar to our study, hematocrit decreased significantly and remained suppressed during long-term therapy (19). A considerable part of intravascular volume expansion, however, occurs within the first hour of nitroglycerin treatment (5), when nitroglycerin effects on large conductance vessels or central filling pressures are in general maximal. Therefore, a significant decrease in hematocrit may be used more as a marker for nitroglycerin treatment than as a marker for tolerance development. In addition, the persistent drop in hematocrit actually lends support to the conclusion that tolerance to epicardial artery effects does not follow the same time line as tolerance in other vascular beds.

Changes in heart rate and mean arterial pressure. Intravenous nitroglycerin infusion in untreated patients caused a dose-dependent decrease in mean arterial pressure and an increase in heart rate. In patients treated with nitroglycerin for 24 h, additional short-term nitroglycerin challenges to the ongoing long-term infusion resulted in similar changes in these systemic hemodynamic variables. A clear-cut attenuation of nitroglycerin effects on systemic hemodynamic variables was observed after a 3-day infusion period. However, tolerance to the blood pressure-lowering effects of organic nitrates has been reported to occur within 24 h of continuous treatment (29). Therefore, our data may indicate that tolerance to systemic hemodynamic effects, which is usually established within 24 h of continuous nitrate therapy, can be overcome by additional nitroglycerin challenges. After 3 days of continuous nitroglycerin treatment, additional short-term nitroglycerin challenges were able to cause a graded response of tolerant large coronary arteries but failed to induce significant changes in heart rate and mean arterial pressure. This phenomenon has also been observed in chronically instrumented dogs (13,28) and may indicate that the nitroglycerin concentrations chosen were sufficient to overcome tolerance in large coronary arteries but may be too small to overcome tolerance in the systemic circulation.

What mechanisms are responsible for attenuation of nitroglycerin action within the 24- and 72-h infusion periods? Numerous studies have documented an early loss (within 24 h) of the nitroglycerin effects during continuous treatment (2,4,16). It remains to be determined whether neurohormonal counterregulatory mechanisms or changes intrinsic to the tolerant vasculature itself play a major causal role in this phenomenon. Our data indicate that within 24 h of continuous nitroglycerin treatment, the vasodilator effects of nitroglycerin on large coronary arteries are preserved even in the presence of neurohormonal adjustments and signs of intravascular volume expansion. Vasopressin and angiotensin II are potent constrictors of small arterioles (30,31), a vessel region that has been demonstrated to be itself nitrate insensitive (32,33). Therefore, it is tempting to speculate that within 24 h of continuous nitroglycerin treatment, the sensitivity of large epicardial arteries to nitroglycerin has not changed, and pseudotolerance rather than true vascular tolerance mechanisms is responsible for the well-known rapid loss of the nitroglycerin vasodilator effects within this time frame. However, within 3 days of continuous nitroglycerin infusion tolerance in large epicardial arteries occurred even in the absence of further neurohormonal activation. This observation indicates a desensitization of coronary conductance vessels to nitroglycerin, which might possibly be related to enhanced destruction of nitric oxide by vascular superoxide anion production (34) or may be secondary to an increase in sensitivity to vasoconstrictors (35) or to oxidation of the enzyme responsible for nitroglycerin biotransformation (36).

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References

1. Abrams J. A reappraisal of nitrate therapy. JAMA 1988;259:396–401.
2. Packer M, Lee W, Kessler PD, Gottlieb SS, Medina N, Yushak M. Prevention and reversal of nitrate tolerance in patients with congestive heart failure. N Eng J Med 1987;317:799–804.

3. Parker JD, Farrell B, Fenton T, Cohanim M, Parker JO. Counter-regulatory responses to continuous and intermittent therapy with nitroglycerin. Circulation 1991;84:2336–45.
4. Zimrin D, Reichek N, Bogin KT, et al. Antianginal effects of intravenous nitroglycerin over 24 hours. Circulation 1988;77:1376–84.
5. Dupuis J, Lalonde G, Lemieux R, Rouleau J. Tolerance to intravenous nitroglycerin in patients with congestive heart failure: role of increased intravascular volume, neurohumoral activation and lack of prevention with N-acetylcysteine. J Am Coll Cardiol 1990;16:923–31.
6. Needleman P, Johnson EMJ. Mechanism of tolerance development to organic nitrates. J Pharmacol Exp Ther 1973;184:709–15.
7. Brien JF, McLaughlin BE, Breedon TH, Bennett BM, Nakatsu K, Marks GS. Biotransformation of glyceryl trinitrate occurs concurrently with relaxation of rabbit aorta. J Pharmacol Exp Ther 1986;237:608–14.
8. Rapoport RM, Waldman SA, Ginsburg R, Molina CR, Murad F. Effects of glyceryl trinitrate on endothelium-dependent and -independent relaxation and cyclic GMP levels in rat aorta and human coronary artery. J Cardiovasc Pharmacol 1987;10:82–9.
9. Münzel T, Kurz S, Holtz J, et al. Neurohormonal inhibition and hemodynamic unloading during prolonged inhibition of ANF degradation in patients with severe chronic heart failure. Circulation 1992;86:1089–98.
10. Drexler H, Zeiher AM, Wollschlager H, Meinertz T, Just H, Bonzel T. Flow-dependent coronary artery dilatation in humans. Circulation 1989;80:466–74.
11. Wollschläger H, Lee P, Zeiher AM, Solzbach U, Bonzel T, Just H. Improvement of quantitative angiography by exact calculation of radiological magnification factors. Comput Cardiol 1986;11:483–6.
12. Hishashi K, Yamamoto H, Noma M, et al. Effects of continuous intravenous infusion of isosorbide dinitrate on development of tolerance to vasodilating action in human epicardial coronary arteries. Am Heart J 1994;128:230–6.
13. Münzel T, Mülsch A, Holtz J, Harrison DG, Bassenge E. Mechanisms of interaction between the sulfhydryl precursor L-methionine and glyceryl trinitrate. Circulation 1992;86:995–1003.
14. Moreyra AE, Kostis JB. Effect of cutaneous nitroglycerin patches on coronary artery diameter: issues concerning development of tolerance. J Am Coll Cardiol 1989;13:428–33.
15. Stewart DJ, Holtz J, Bassenge E. Long-term nitroglycerin treatment: effect on direct and endothelium-mediated large coronary artery dilation in conscious dogs. Circulation 1987;75:847–56.
16. Elkayam U, Kulick D, McIntosh N, Roth A, Hsueh W, Rahimtoola SH. Incidence of early tolerance to hemodynamic effects of continuous infusion of nitroglycerin in patients with coronary artery disease and heart failure. Circulation 1987;76:577–84.
17. Parker JO, Fung HL, Ruggirello D, Stone JA. Tolerance to isosorbide dinitrate: rate of development and reversal. Circulation 1983;68:1074–80.
18. Thadani U, Hamilton SF, Olson E, et al. Transdermal nitroglycerin patches in angina pectoris. Ann Intern Med 1986;105:485–92.
19. Parker JD, Parker JO. Effects of therapy with an angiotensin converting enzyme inhibitor on hemodynamic and counterregulatory responses during continuous therapy with nitroglycerin. J Am Coll Cardiol 1993;21:1445–53.
20. Mehra A, Ostrzega E, Shotan A, Johnson JV, Elkayam U. Persistent hemodynamic improvement with short-term nitrate therapy in patients with chronic congestive heart failure already treated with captopril. Am J Cardiol 1992;70:1310–4.
21. Muiesan ML, Boni E, Castellano M, et al. Effects of transdermal nitroglycerin in combination with an ACE-inhibitor in patients with chronic stable angina pectoris. Eur Heart J 1993;14:1701–8.
22. Katz RJ, Levy WS, Buff L, Wasserman AG. Prevention of nitrate tolerance with angiotensin-converting enzyme inhibitors. Circulation 1991;83:1271–7.
23. Feldman RL, Pepine CJ, Conti CR. Magnitude dilation of large and small coronary arteries by nitroglycerin. Circulation 1981;64:324–33.
24. Pagani M, Vatner SF, Braunwald E. Hemodynamic effects of intravenous nitroprusside in the conscious dog. Circulation 1978;57:144–51.
25. Ueda K, Sakai M, Matsuhita S, Kuwajiama I, Murakami M. Effect of orally administered hydralazine on neurohormonal factors and hemodynamic response in aged patients with chronic congestive heart failure. Jpn Heart J 1983;24:711–21.
26. Münzel T, Bassenge E. Chronic angiotensin converting enzyme inhibition with high dose enalapril retards nitrate tolerance development in large epicardial arteries and prevents coronary rebound constriction in vivo. Circulation. In press.
27. Stewart DJ, Elsner D, Sommer O, Holtz J, Bassenge E. Altered spectrum of nitroglycerin action in long-term treatment: nitroglycerin-specific venous tolerance with maintenance of arterial vasodepressor potency. Circulation 1986;74:573–82.
28. Münzel T, Holtz J, Mülsch A, Stewart D, Bassenge E. Nitrate tolerance in epicardial arteries or in the venous system is not reversed by N-acetylcysteine in vivo, but tolerance-independent interactions exist. Circulation 1989;79:188–97.
29. Thadani U, Manyari D, Parker O, Fung HL. Tolerance to the circulatory effects of oral isosorbide dinitrate. Circulation 1980;61:526–35.
30. Khayal MA, Eng C, Franzen D, Breall JA, Kirk ES. Effects of vasopressin on the coronary circulation: reserve and regulation during ischemia. Am J Physiol 1985;248:H516–22.
31. Cohen MV, Kirk ES. Differential response of large and small coronary arteries to nitroglycerin and angiotensin. Circ Res 1973;33:445–53.
32. Sellke FW, Tomanek RJ, Harrison DG. L-Cysteine selectively potentiates nitroglycerin-induced dilation of small coronary microvessels. J Pharmacol Exp Ther 1991;258:365–9.
33. Sellke FW, Myers PR, Bates JN, Harrison DG. Influence of vessel size on the sensitivity of porcine microvessels to nitroglycerin. Am J Physiol 1990;258:H515–20.
34. Münzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance: a novel mechanism of tolerance and cross tolerance. J Clin Invest 1995;95:187–94.
35. Münzel T, Kurz S, Giaid A, Stewart D, Harrison DG. Evidence for a role of endothelin-1 and protein kinase C in nitrate tolerance. Proc Natl Acad Sci USA 1995;92:5244–8.
36. Seth P, Fung HL. Biochemical characterization of a membrane-bound enzyme responsible for generating nitric oxide from nitroglycerin in vascular smooth muscle cells. Biochem Pharmacol 1993;46:1481–6.